PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12P 7/62, 7/42

A1

(11) International Publication Number: **WO 99/60151**

(43) International Publication Date: 25 November 1999 (25.11.99)

(21) International Application Number: PCT/IB99/00923

(22) International Filing Date: 21 May 1999 (21.05.99)

(30) Priority Data:
P-9800144 21 May 1998 (21.05.98) SI

(71) Applicant *(for all designated States except US)*: LEK PHARMACEUTICAL AND CHEMICAL COMPANY D.D. [SI/SI]; Verovskova 57, 1526 Ljubljana (SI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KRANJC, Sašo [SI/SI]; Lek Pharmaceutical and Chemical Company, d.d., Verovskova 57, 1526 Ljubljana (SI). IVANC, Irena [SI/SI]; Lek Pharmaceutical and Chemical Company, d.d., Verovskova 57, 1526 Ljubljana (SI). SCHAUER, Manica [SI/SI]; Lek Pharmaceutical and Chemical Company, d.d., Verovskova 57, 1526 Ljubljana (SI).

(74) Agent: OSER, Andreas; Tiedtke–Bühling–Kinne, Bavariaring 4, D–80336 München (DE).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NEW BIOTECHNOLOGICAL PROCESS FOR PREPARING HYDROXYLATED ML–236B DERIVATIVES, KNOWN AS M–4 AND M–4', AND ANALOGUES THEREOF

(57) Abstract

The very effective conversion of ML–236B substances and derivatives thereof into 6'–hydroxylyted products with the microorganisms of species *Amycolatopsis orientalis*, or with an extract or a hydroxylation–effective enzyme derived from said microorganism, is described. The products obtained are suitable as HMG–CoA reductase inhibitors or intermediates thereof. Thus, the products can be used, for example, as an antihypercholesterolemic in pharmacy.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Title

New Biotechnological Process for Preparing Hydroxylated ML-236 B Derivatives, known as M-4 and M-4', and analogues thereof

Technical Field

The present invention relates to a novel process for preparing hydroxylated ML-236 B derivatives, known as M-4 and M-4', and analogues thereof, in particular to an enzymatic hydroxylation by means of a microbiological process.

Prior Art

ML-236B and derivatives thereof as well as analogues are known as HMG-CoA reductase inhibitors and are disclosed in GB Pat. No. 1,555,831. They are produced by fermentation with various microorganisms of the genera Gilbertella, Streptomyces, Circinella, Monascus, Nocardia, Amycolata, Mucor or Penicillium (as disclosed in US Pat. No. 4,346,227 and US Pat. No. 4,537,859). The hydroxylated forms of ML-236B, its derivatives and analogues, especially those

known as M-4 and M-4', have been found to be substantially effective inhibitors of HMG-CoA reductase, and their mixture in the form of a pharmaceutically acceptable salt such as the sodium salt has a therapeutic value as an antihypercholesterolemic agent. All substances exist in the form of an acid or a lactone.

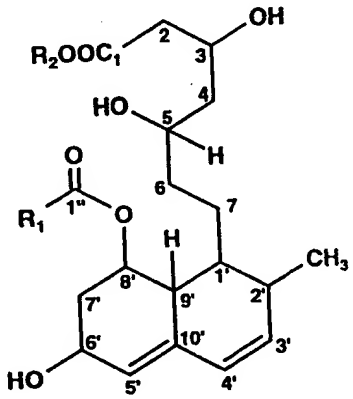
Conventionally, the preparation of pharmaceutically acceptable salts of the HMG-CoA reductase inhibitors, such as the M-4 and M-4' substances, is a feed-batch process. The first part comprises preparation of a ML-236B substance by fermentation with microorganisms of the genus Penicillium and its isolation using conventional isolation techniques and preparation of the sodium salt thereof as described in US Pat. No. 4,137,322. The second part comprises cultivation of a microorganism of one of the aforementioned genera in the medium to which ML-236B, typically in the form of sodium salt, is added, isolation of the M-4 and M-4'substances and optionally preparation of pharmaceutically acceptable salts thereof as disclosed in US Pat. No. 4,346227 and US Pat. No. 4,537,859.

Object of the Invention

There exists a constant need for a novel and improved biotechnological process which will enable the most effective conversion of a ML-236B substance and derivatives and analogues thereof into their hydroxylated forms. The higher yield in conversion into the hydroxylated forms is also important because of high price of the starting substance.

Description of the Invention and the Preferred Embodiments

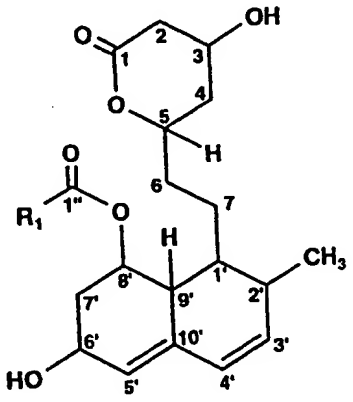
The object of the invention has been solved by the provision of a process for preparing a compound defined by the following formula (I)



(I),

wherein R_1 represents substituted or unsubstituted alkyl or substituted or unsubstituted aryl, and R_2 independently represents H, substituted or unsubstituted alkyl or a cation;

or the corresponding lactone (II)



(II),

wherein R_1 is as defined above;

which process comprises contacting a 6'-H analogue of the above formula (I) or (II) for hydroxylation with a microorganism of the species *Amycolatopsis orientalis* or with an extract or a hydroxylation-effective enzyme derived from said microorganism.

According to the present invention a specific microorganism was found which, being used in a microbiological process or in the form of an extract or a hydroxylation-effective enzyme derived from said microorganism, converts desired compound substrates, namely the 6'-H analogue of the compounds of formula (I) or the lactone of formula (II), into their hydroxylated forms with improved, relatively high yields. Various microorganisms reported in the literature as potential producers of the hydroxylated forms have been studied, and it has surprisingly been found that the best results are obtained when employing the microorganism of *Amycolatopsis orientalis* and expecially when employing the strain ATCC 19795, or the extract or the hydroxylation-effective enzyme derived therefrom. *Amycolatopsis orientalis* is to date merely known as a producer of antibiotic vancomycin.

The strain *Amycolatopsis orientalis* ATCC 19795 is characterized by a well-branched mycelium which under specified conditions fragments into shorter particles. Colonies grown on a solid agar culture medium are of a pale blue colour, velvet-like appearance, slightly folded and upheaved, 5 to 10 mm in diameter. The most convenient temperature for growth is within the range from 24 °C to 30 °C, with a notion that this microorganism poorly sporulates.

The subject of the present invention is the enzymatic hydroxylation process which can be suitably effected by contacting the aforementioned 6'-H analogue of the compound

of formula (I) or the corresponding lactone (II) with the microorganism *Amycolatopsis orientalis*, especially the strain deposited as ATCC 19795, in a suitable fermentation medium.

A fermentation medium should preferably comprise a source of assimilable carbon, such as glucose, saccharose, dextrins, glycerol, starch, soybean oil and molasses, a source of assimilable nitrogen, such as soybean flour, meat and yeast extracts, peptones, ammonium salts and, if required, various inorganic salts, such as sodium chloride, potassium chloride, magnesium sulphate, calcium carbonate and phosphates. Most conventional culture media may be used that employ microorganisms of the genus *Amycolatopsis* for fermentation. Suitable inoculation and fermentation media are, for example, described by J.J. McIntyre et al. in Biotechnology and Bioengineering, vol. 49, pp. 412-420 (1996); L.D. Boeck et al. in The Journal of Antibiotics, Vol. XXXVII No. 5, pp. 446-453 (1984); G.J. Clark et al. in Microbiology, Vol. 141, Pt. 3, pp. 663-669 (1995); and in US Patent No. 4,547,488; all descriptions being incorporated herein by way of reference.

Fermentation is carried out under aerobic conditions and at a temperature within a suitable range, for example from 20° to 36 °C, preferably from 24 °C to 30 °C. The aforementioned substrate compound to be hydroxylated may be contacted with the microorganism, preferably in the form of sodium salt, at any time in the course of fermentation or after completion of the fermentation, but an addition at the vegetative part of fermentation, which is normally between 24 and 48 hours after beginning cultivation, is particularly preferred. The compound substrate is added to a total final concentration of 0.01 wt.-% to 5 wt.-%, preferably 0.05 wt.-% to 0.5 wt.-%, based on the total fermentation broth weight or the contacting liquid. Addition to a fermentation medium may be

either batch, feed-batch or continuous. Fermentation is usually completed within 2 to 5 days after addition of the substrate compound. Then, the microorganism cells are separated, preferably by filtration, and the resulting filtrate is extracted with an organic solvent which is preferably water immiscible or has a limited miscibility with water, such as ethyl acetate, ether (for example diethyl ether) or chlorotorm. Subsequently, the organic solvent is removed, suitably by evaporation, and if desired the resulting crude product is subjected to further conventional purification and isolation processes, for example employing column chromatography, e.g. using silica gel column, and eluting the desired compound with an appropriate eluent. If required, the resulting product may be subjected to recrystallization, salification (if it is desired in the form of salt), lactonization or esterification, with methods known to those skilled in the art.

If the contacting step is carried out after completion of the fermentation such as about 4 to 5 days after beginning the cultivation, the microorganism cells can be collected, for example by filtration, optionally washed with an appropriate buffer solution such as phosphate buffer, and then contacted with the substrate compound in an appropriate buffer solution (pH from 5 to 9 at a temperature suitably from 20 to 45 °C, preferably from 25 to 30 °C).

Alternatively, the hydroxylation process can also be effected by a cell-extract of *Amycolatopsis orientalis*. The cell-extract may be used as it is, or it may be used in a modified form, e.g. previously drying it or separating non-hydroxylation effective cell constituents. Furthermore, an hydroxylation-effective enzyme derived from said microorganism can be employed for the hydroxylation process. For example, an enzyme being effective for the hydroxylation step is previously isolated from *Amycolatopsis orientalis*.

Also contemplated within the concept of employing an enzyme derived from the microorganism is the use of a modified enzyme, for example a stabilized enzyme or an enzyme being bound to a support, and the use of a genetically engineered enzyme corresponding to the hydroxylation-effective enzyme derived from said microorganism. Suitable methods for obtaining the cell-extract or hydroxylation-effective enzyme, or their modified forms, are well known to those skilled in the art.

The product obtained by the microbiological process is typically a mixture of both 6'-hydroxy α- and ß-configurations and can be used as such. If desired, the isomers with the respective α- and ß-configuration may be separated by an appropriate isomer resolution technique known to those skilled in the art.

The structural moieties of R_1 and R_2 can be selected from the definitions as specified above for obtaining HMG-CoA reductase inhibitors or intermediates thereof.
Thus, R_1 may represent optionally substituted alkyl or optionally substituted aryl. The alkyl group include straight chain, branched or cyclic hydrocarbon optionally having unsaturated double bonds and optionally being substituted. The main chain of the alkyl group has 1 to 15, preferably 1 to 10 and more preferably 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, hexyl, the isomers and the branched derivatives thereof, and the like. The optional substituents include one or more of the group consisting of halogen such as chloro, amino, lower alkyl amino such as mono- or dimethylamino, hydroxy, alkoxy, cyano, nitro, and the like. A preferred substituent is hydroxy. If present, a branching hydrocarbon group preferably has 1 to 4 carbon atoms, such as methyl and ethyl. Possible aryl residues include substituted or unsubstituted phenyl, biphenyl and naphthyl,

wherein the optional substituents are selected from the group consisting of lower alkyl, alkoxy, halogen such as chloro, amino, lower alkyl amino such as mono- or dimethylamino, hydroxy, alkoxy, cyano, nitro, and the like.

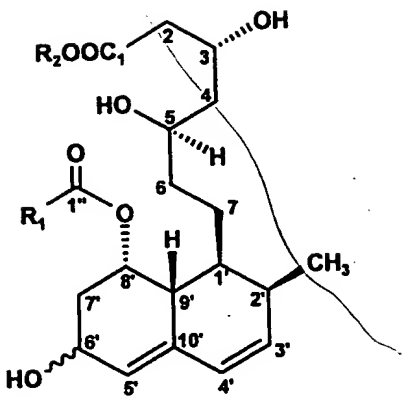
It is particularly preferred that R_1 represents an alkyl residue having the following structure:

$$H_3C—CHR_4—C(CH_3)R_3—\quad ,$$

wherein R_3 denotes H or CH_3 and R_4 independently denotes H or OH.

Furthermore, R_2 may represent H, alkyl or a cation, corresponding to the presence of a free acid, an ester or a salt of the compound of formula (I), respectively. Possible alkyl residues include straight chain, branched or cyclic hydrocarbon optionally having unsaturated double bonds and optionally being substituted, as previously described with respect to R_1. Possible cations include metal cations, for example sodium, potassium, lithium, calcium, magnesium, preferably alkali metal cations such as sodium, an ammonium group, an alkyl ammonium group, and the like.

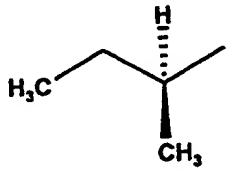
In order to provide a more potent HMG-CoA reductase inhibitor, the prepared compound of formula (I) has the following configuration (Ia):



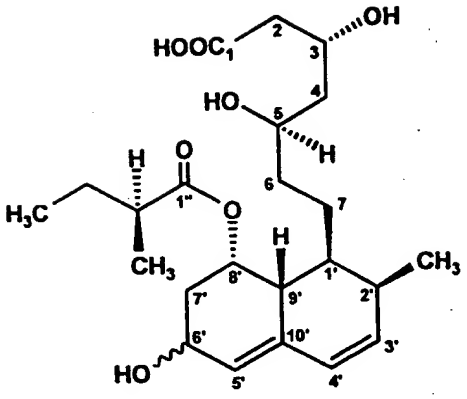
(Ia),

or the corresponding lactone compound (IIa); wherein R_1 and R_2 are as defined above, and the 6' ~~~ OH-group represents an α- or a β-configuration, or a mixture of both α- and β-configurations. The compounds of formula (Ia) or (IIa) are obtained by starting with a 6'-H analogue having the corresponding configuration.

A particularly preferred example for R_1 is an alkyl residue having the following structure and configuration:



In a specifically preferred embodiment of the present invention, a compound having the following formula (III) or the salt thereof or the corresponding lactone is prepared in order to provide a particularly active and potent HMG-CoA reductase inhibitor or an intermediate thereof:



(III),

wherein the 6' ~~~ OH-group represents an α- or a β-configuration, or a mixture of both α- and β-configurations. If the salt is prepared, it is typically the pharmaceutically acceptable salt, in particular the sodium salt.

For obtaining the starting compounds for the process of the present invention, and for preparing the derivatives, analogues and variations (with respect to the definitions of R_1 and R_2) such as the lactone form, the ester form or the salt form from the obtained 6'-hydroxylated product, reference is made to the descriptions of GB Patent No. 1,555,831, US Pat. No. 4,346,227 and US Pat. No. 4,537,859.

The compounds obtained by the process according to the present invention can be further modified by synthetic, semi-synthetic or biochemical procedures. Thus, the compounds obtained in the present invention may themselves constitute intermediates for producing further HMG-CoA reductase inhibitors. For example, one or both double bonds in the ring structure of the obtained compounds of formula (I) or (II) may be hydrogenated.

The HMG-CoA reductase inhibitors obtained by the process according to the present invention, which are preferably purified to at least 99.5%, more preferably 99.6 %, can be beneficially used for the preparation of a pharmaceutical

for the prevention and/or treatment of diseases. The compounds obtained by the process according to the present invention, or the HMG-CoA reductase inhibitors derived therefrom, can be effectively used as an antihypercholesterolemic agent. The compounds can therefore be used for the preparation of a medicament for the control of cholesterol in the body of an individual. They can further be used for the prevention or the treatment of atherosclerosis. The obtained inhibitors and pharmaceuticals are particularly useful as preventives for reducing the risk of stroke, transient ischemic attack and myocardial infarction.

This invention is illustrated but in no way limited by the following examples.

Example 1

Preparation of the inoculum

The rationale for determining the most suitable productive culture was selection of individual colonies with the typical morphological characteristics. The selected colonies were transferred to a sterile potter to agar slopes and homogenised. The resulting colonies were transferred and incubated in the thermostat at 26 °C to 30 °C for 7 to 14 days. During that time surfaces of agar slopes were overgrown by a culture of homogeneous, folded, smooth, white to pale greyish-blue mycelium. A portion (0.5 to 1 ml) of the resulting culture was then inoculated into a vegetative medium.

Agar medium for preparation of agar slopes and petri plates:

Raw material	Amount
Dextrin	10 g
Consumer's glucose	5 g
Casaminic acid	3 g
Yeast extract	4 g
$CaCO_3$	1 g
Agar	15 g
Sterile water	up to 1000 ml

No pH adjustment needed.

Vegetative phase of fermentation

The inoculum grown on agar slope at 26 °C to 30 °C for 10 days and prepared according to the method described in Example 1, was inoculated on a 500-ml Erlenmeyer flask containing 50 ml of the vegetative medium. After 2 days a portion of the culture (5 to 10%) was transferred onto the fermentation medium.

Vegetative medium

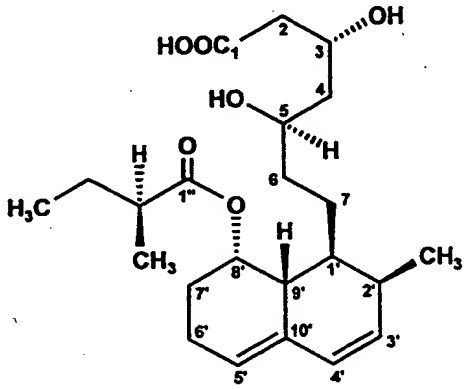
Raw material	**Amount**
Corn starch for fermentation	20 g
Soybean flour for fermentation	14 g
Glucose	3 g
Yeast extract	5 g
NaH_2PO_4 x 2 H_2O	3.3 g

Tap water to 1000 ml

Example 2

Conversion of a ML-236B substance into 6'-hydroxy derivatives M-4 and M-4' thereof

A portion of the culture, prepared according to the method described in Example 1, was transferred into Erlenmeyer flasks with fermentation medium 1 to which a ML-236B substance having the formula shown below, in the form of sodium salt, was added to a concentration of 200 mg/L on the second day of fermentation. Analyses of the concentration of the 6'-hydroxylated M-4 and M-4' compounds in the fermentation broth showed the total final concentration of the M-4 and M-4' substances in the fermentation broth to be 60 mg/ml after 3 days of fermentation at temperature between 24 °C and 30 °C.



Fermentation medium 1

Raw material	Amount
Glycerol	80 g
Soybean flour	20 g
Soybean oil	0.1 g
Calcium gluconate	12 g
Magnesium chloride x 6 H_2O	1.3 g
Magnesium sulphate x 7 H_2O	1 g

$NaNO_3$	10 g

Tap water to 1000 ml

All raw materials were dissolved in tap water, the pH was then adjusted to a value of 7.8 by addition of 1M aqueous solution of NaOH. The resulting medium was poured into 300 ml-Erlenmeyer flasks, 30 ml per flask.

Example 3

Conversion of a ML-2368 substance into 6'-hydroxy derivatives M-4 and M-4 thereof

A portion of the culture, prepared according to the method described in Example 1, was transferred into Erlenmeyer flasks with fermentation medium 3 to which ML-236B, in the form of sodium salt, was added to a concentration of 400 mg/L on the second day of fermentation. Analyses of the concentration of M-4 and M-4' substances in the fermentation broth showed the total final concentration of the M-4 and M-4' substances in the fermentation broth to be 160 mg/ml after 3 days of fermentation at temperature between 24 °C and 30 °C, indicating a 40% conversion.

Fermentation medium 3

Raw material	**Amount**
Glycerol	20 g
Soybean flour	20 g
Calcium gluconate	12 g
Magnesium chloride x 6 H_2O	1.3 g
Magnesium sulphate x 7 H_2O	1 g
$NaNO_3$	10 g

Tap water to 1000 ml

All raw materials were dissolved in tap water, the pH was then adjusted to a value of 7.8. The resulting medium was poured into 300 ml-Erlenmeyer flasks, 30 ml per flask.

Example 4

Conversion of a ML-236B substance into 6'-hydroxy derivatives M-4 and M-4' thereof at pilot plant scale

The contents of ten Erlenmeyer flasks with the culture, prepared according to the method described in Example 1, whereat the vegetative part of fermentation was shorten to 24 hours, were used to inoculate the fermenter (14 l) with 10 l of fermentation medium. After 24-hour fermentation, ML-236B, in the form of sodium salt (470 mg of ML-236B dissolved in 500 ml of water) was continually added to the medium for 6 hours. Analyses of the concentration of M-4 and M-4' substances in the fermentation broth showed the total final concentration of the M-4 and M-4' substances in the fermentation broth to be 300 mg after 36 hours of fermentation at temperature between 24 °C and 30 °C, indicating a 64% conversion.

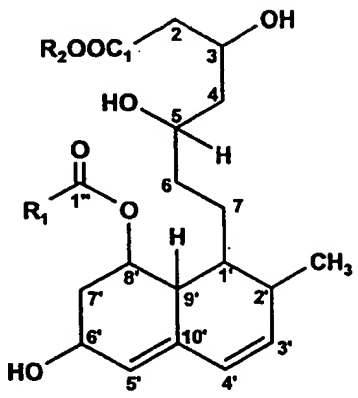
Fermentation medium 2

Raw material	**Amount**
Glycerol	20 g
Corn starch for fermentation	20 g
Soybean flour for fermentation	14 g
Glucose	10 g
Yeast extract	5 g
NaH_2PO_4 x 2 H_2O	3.3 g
Tap water to 1000 ml	

All raw materials were dissolved in tap water, the pH was then adjusted to a value of 7.8.

Claims

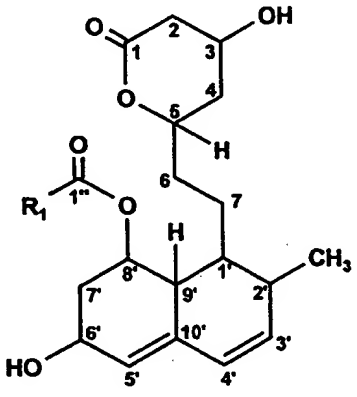
1. A process for preparing a compound defined by the following formula (I)



(I),

wherein R_1 represents substituted or unsubstituted alkyl or substituted or unsubstituted aryl, and R_2 independently represents H, substituted or unsubstituted alkyl or a cation;

or the corresponding lactone (II)

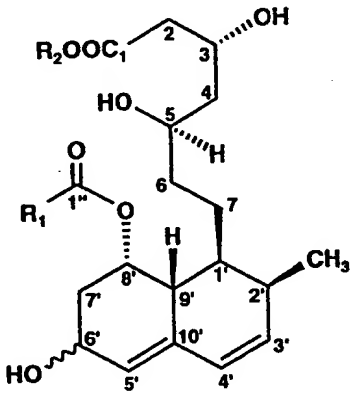


(II),

wherein R_1 is as defined above;

which process comprises contacting a 6'-H analogue of the above formula (I) or (II) for hydroxylation with a microorganism of the species *Amycolatopsis orientalis* or with an extract or a hydroxylation-effective enzyme derived from said microorganism.

2. A process according claim 1, wherein the prepared compound of formula (I) has the following configuration (Ia):



(Ia),

or the corresponding lactone compound (IIa);
wherein R_1 and R_2 are as defined in claim 1, the 6' ~~~ OH-group represents an α- or a β-configuration, or a mixture of both α- and β-configurations.

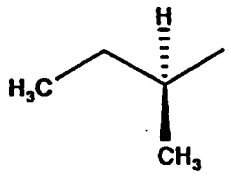
3. A process according to claim 1 or 2, wherein R_2 represents H, a C_1-C_6 alkyl group or a cation selected from the group consisting of an alkali metal, an ammonium group or an alkyl ammonium group.

4. A process according to any one of claims 1 to 3, wherein R_1 represents an alkyl residue having the following structure:

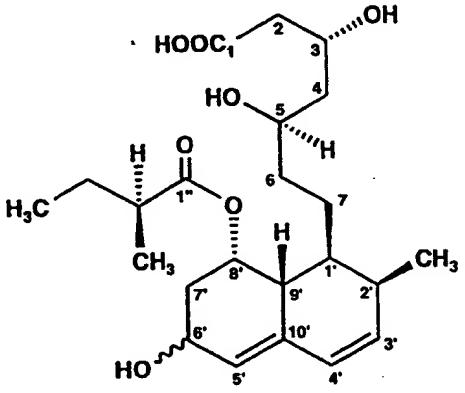
$H_3C—CHR_4—C(CH_3)R_3—$,

wherein R_3 denotes H or CH_3 and R_4 independently denotes H or OH.

5. A process according to claim 4, wherein said alkyl residue has the following structure and configuration:



6. A process according to claim 1, wherein a compound having the following formula (III) or the salt thereof or the corresponding lactone is prepared:



(III),

wherein the 6' ~~~ OH-group represents an α- or a β-configuration, or a mixture of both α- and β-configurations.

7. A process according to any one of the preceding claims, wherein said microorganism is *Amycolatopsis orientalis* ATCC 19795.

8. A process according to any one of the preceding claims, wherein said contacting step is performed in the course of fermentation of said microorganism, and wherein said 6'-H analogue is added to the fermentation broth in a batch, feed-batch or continuous manner.

9. A process according to claim 8, wherein said 6'-H analogue is added to the fermentation medium to the final content of 0,05 to 0,5 wt.-%, based on the total medium weight.

10. A process according to claim 8, wherein sources of assimilable carbon, nitrogen and/or phosphorus is/are added during the fermentation.

11. A process according to any one of the preceding claims which is used for the preparation of a HMG-CoA reductase inhibitor.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IB 99/00923

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P7/62 C12P7/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 153 124 A (SANKYO COMPANY LTD.) 6 October 1992 see the whole document	1-11
X	EP 0 649 907 A (BRISTOL-MYERS SQUIBB) 26 April 1995 see page 2 - page 8; claims 1-6	1-11
X	US 4 346 227 A (SANKYO COMPANY LTD.) 24 August 1982 cited in the application see the whole document	1-11
Y	GHERNA, R. AND PIENTA, P.: "ATCC-Catalogue of Bacteria and Phages." 1992 , AMERICAN TYPE CULTURE COLLECTION , ROCKVILLE, MARYLAND/USA XP002109634 see page 26	1-11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1999	03/08/1999

Name and mailing address of the ISA	Authorized officer °
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Douschan, K

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intr Ional Application No
PCT/IB 99/00923

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 537 859 A (SANKYO COMPANY LTD.) 27 August 1985 cited in the application see the whole document	1-11

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/IB 99/00923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5153124 A	06-10-1992	AT 69602 T	15-12-1991
		DE 3682557 A	02-01-1992
		EP 0215665 A	25-03-1987
		JP 1696791 C	28-09-1992
		JP 3066297 B	16-10-1991
		JP 62174040 A	30-07-1987
		US 5272174 A	21-12-1993
		JP 1790339 C	29-09-1993
		JP 4082135 B	25-12-1992
		JP 62190094 A	20-08-1987
EP 0649907 A	26-04-1995	AU 679113 B	19-06-1997
		AU 7597294 A	11-05-1995
		CA 2134025 A	23-04-1995
		CN 1106067 A	02-08-1995
		FI 944926 A	23-04-1995
		HU 69959 A	28-09-1995
		IL 111084 A	04-01-1998
		JP 7184670 A	25-07-1995
		SG 47504 A	17-04-1998
US 4346227 A	24-08-1982	JP 1347361 C	13-11-1986
		JP 57002240 A	07-01-1982
		JP 61013699 B	15-04-1986
		JP 1500955 C	28-06-1989
		JP 57108039 A	05-07-1982
		JP 63048858 B	30-09-1988
		JP 1454452 C	25-08-1988
		JP 57050894 A	25-03-1982
		JP 62054476 B	16-11-1987
		JP 1475008 C	18-01-1989
		JP 57067575 A	24-04-1982
		JP 63021672 B	09-05-1988
		AT 374495 B	25-04-1984
		AT 256781 A	15-09-1983
		AU 549988 B	27-02-1986
		AU 7137681 A	10-12-1981
		BE 889150 A	09-12-1981
		CA 1150170 A	19-07-1983
		CH 655090 A	27-03-1986
		DE 3122499 A	24-12-1981
		DK 247081 A,B,	07-12-1981
		FI 811762 A,B,	07-12-1981
		FR 2483912 A	11-12-1981
		GB 2077264 A,B	16-12-1981
		IE 51270 B	26-11-1986
		MX 9203563 A	01-09-1992
		NL 8102737 A,B,	04-01-1982
		SE 453389 B	01-02-1988
		SE 8103560 A	07-12-1981
		US 4410629 A	18-10-1983
		US 4448979 A	15-05-1984
US 4537859 A	27-08-1985	JP 1702757 C	14-10-1992
		JP 3071116 B	12-11-1991
		JP 58089191 A	27-05-1983
		AT 387585 B	10-02-1989
		AT 425182 A	15-07-1988

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/IB 99/00923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4537859 A		AU 551720 B	08-05-1986
		AU 9061082 A	26-05-1983
		BE 895080 A	16-03-1983
		CA 1186647 A	07-05-1985
		CH 651065 A	30-08-1985
		DE 3242849 A	01-06-1983
		DK 516182 A,B,	21-05-1983
		FI 823978 A,B,	21-05-1983
		FR 2516935 A	27-05-1983
		GB 2111052 A,B	29-06-1983
		NL 8204505 A	16-06-1983
		SE 453996 B	21-03-1988
		SE 8206580 A	21-05-1983
		ZA 8208535 A	26-10-1983